Claims

1. A method for analyzing the C-terminal amino acid sequence of a peptide to be examined, which method comprises steps of:

preparing a mixture containing a series of reaction products obtained by process for releasing the C-terminal amino acids successively from the target peptide to be examined by chemical procedure,

analyzing the difference of molecular weight between said series of reaction products and the original peptide by means of mass spectrometry to measure the decreases in molecular weight associated with the successive release of the C-terminal amino acid, and

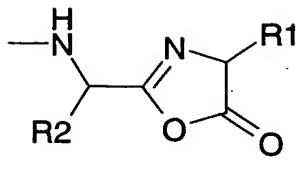
identifying a series of the amino acids removed successively, based on a series of the measured decreases in molecular weight and arranging those in sequence from the C-terminus to obtain the information of the C-terminal amino acid sequence,

wherein in the step of releasing the C-terminal amino acids successively, the treatment for the sample of the target peptide that has been subjected to separation by gel electrophoresis and is maintained in a state that it is bound on a gel carrier is made through the following process that is conducted in a

state that the sample of the target peptide is kept on the gel carrier, which process comprises steps of:

removing the water solvent impregnated into the gel carrier by dilution with use of a polar aprotic solvent having no solvency for the gel substance and having affinity for water, to conduct a dehydration treatment for the gel carrier,

immersing, at a temperature selected in a range of 30 ºC to 80 ºC, the gel carrier on which the target peptide sample is still bound after said dehydration treatment in a mixed solution of an alkanoic acid anhydride added with a small amount of a perfluoroalkanoic acid in relative ratio thereto dissolved in a dipolar aprotic solvent that is capable of infiltrating into the gel substance and keeping it in a swollen state, to allow the alkanoic acid anhydride and the perfluoroalkanoic acid to act on the target peptide sample being kept in the bound state; thereby, successive release of the C-terminal amino acids results from the reaction process with use of the mixed solution in which formed is a 5-oxazolone-ring structure represented by the following general formula (III):



(III)

wherein R1 is a side chain of the C-terminal amino acid of the peptide and R2 is a side chain of the amino acid residue positioned just before the C-terminal amino acid, followed by the cleavage of the 5-oxazolone-ring, and

removing the mixed solution used in the reaction for successive release of C-terminal amino acids, by dilution with use of a polar aprotic solvent having no solvency for the gel substance and having affinity for the perfluoroalkanoic acid and the alkanoic acid anhydride as well as the dipolar aprotic solvent, to conduct termination of the releasing reaction and removal of the reaction reagents therefor; and wherein the mixture containing the original peptide and a series of reaction products, which is obtained by conducting said process for successive release of C-terminal amino acids in a state that the sample is bound on a gel carrier, is subjected to the above-mentioned mass spectrometry step.

2. A method claimed in Claim 1, wherein a symmetric anhydride of an alkanoic acid having 2 to 4 carbon

atoms is used as the alkanoic acid anhydride contained in said mixed solution where a small amount of a perfluoroalkanoic acid in relative ratio to the alkanoic acid anhydride is dissolved.

3. A method claimed in Claim 2, wherein a symmetric anhydride of a linear-chain alkanoic acid having 2 to 4 carbon atoms is used as said symmetric anhydride of an alkanoic acid having 2 to 4 carbon atoms.

4. A method claimed in Claim 1, wherein acetic anhydride is used as the alkanoic acid anhydride contained in the mixed solution where a small amount of a perfluoroalkanoic acid in relative ratio to the alkanoic acid anhydride is dissolved.

5. A method claimed in Claim 1, wherein a perfluoroalkanoic acid of which a pKa is in the range of 0.3 to 2.5 is used as the perfluoroalkanoic acid contained in the mixed solution where a small amount of the perfluoroalkanoic acid in relative ratio to the alkanoic acid anhydride is dissolved.

6. A method claimed in Claim 1, wherein a perfluoroalkanoic acid having 2 to 4 carbon atoms is used as the perfluoroalkanoic acid contained in the mixed solution where a small amount of the perfluoroalkanoic acid in relative ratio to the alkanoic acid anhydride is dissolved.

7. A method claimed in Claim 6, wherein a linear-chain perfluoroalkanoic acid having 2 to 4 carbon atoms is used as the perfluoroalkanoic acid having 2 to 4 carbon atoms.

8. A method claimed in Claim 1, wherein in the mixed solution where a small amount of the perfluoroalkanoic acid in relative ratio to the alkanoic acid anhydride is dissolved, the content ratio of the alkanoic acid anhydride and the perfluoroalkanoic acid is selected in the range of 1 to 20 volumes of the perfluoroalkanoic acid per 100 volumes of the alkanoic acid anhydride.

9. A method claimed in Claim 1, wherein, in said reaction treatment in the mixed solution using a dipolar aprotic solvent, said reaction system therefor is kept in a dry atmosphere wherein not only water but also oxygen have been eliminated.

10. A method claimed in Claim 1, wherein, in said process of successive release of C-terminal amino acids, after the step of removing the mixed solution by dilution with use of the polar aprotic solvent to conduct termination of the release reaction and removal of the reaction reagents therefor, there is provided additional step for hydrolysis treatment and then redehydration treatment, in which step

the hydrolysis treatment for said mixture comprising a series of reaction products obtained by the reaction for successive release of C-terminal amino acids is conducted by immersing the gel carrier in an aqueous solution dissolving a basic nitrogen-containing aromatic compound or a tertiary amine compound therein to allow a water molecule to act, in the presence of said basic nitrogen-containing organic compound, on said peptides of the reaction products being still bound on the gel carrier; and then,

the redehydration treatment for the gel carrier is performed by removing said aqueous solution infiltrated into the gel carrier by dilution with use of a polar aprotic solvent having no solvency for the gel substance and having affinity for water.

11. A method claimed in Claim 1, wherein, in said process of successive release of C-terminal amino acids, prior to said reaction treatment in the mixed solution using the dipolar aprotic solvent, there is provided a further step of pretreatment for the target peptide sample that is still bound on the gel carrier after carrying out said step for dehydration treatment, in which step

applying N-acylation protection by the acyl group derived from the alkanoic acid constituting said

alkanoic acid anhydride, to the N-terminal amino group of the target peptide with use of a solution of an alkanoic acid anhydride dissolved in a dipolar aprotic solvent that is capable of infiltrating into the gel substance and keeping it in a swollen state is conducted by immersing, at a temperature selected in a range of 30 ºC to 80 ºC, the gel carrier in the solution of the alkanoic acid anhydride to allow the alkanoic acid anhydride to act on the target peptide sample that is kept in the bound state; and then

removal of said solution is carried out by dilution with use of a polar aprotic solvent having no solvency for the gel substance and having affinity for the alkanoic acid anhydride as well as the dipolar aprotic solvent, to conduct termination of the N-acylation reaction and removal of the reaction reagent therefor.

12. A method claimed in Claim 11, wherein the same alkanoic acid anhydride is employed for the alkanoic acid anhydride used for applying N-acylation protection to the N-terminal of peptide in the pretreatment step, as well as for the alkanoic acid anhydride used in the subsequent step of successive release of C-terminal amino acids.

13. A method claimed in Claim 1, wherein preparation

of the target peptide sample that has been subjected to separation by gel electrophoresis and is maintained in a state that it is bound on a gel carrier is carried out by means of electrophoresis using a polyacrylamide gel as the gel carrier.

14. A method claimed in Claim 1, wherein, in said step of analyzing the series of reaction products and the original peptide by mass spectrometry, a MALDI-TOF type mass spectrometry is selected as the mass spectrometry.